Int J Clin Exp Pathol 2019;12(7):2657-2664
www.ijcep.com /ISSN:1936-2625/IJCEP0095369

Original Article

H3K27 trimethylation and H3K9 dimethylation as poor prognostic markers for patients with esophageal squamous cell carcinoma

Shaofeng Lin[1,2], Menghan Zhou[3,4], Yiping Li[5], Yanping Chen[6], Weizhang Xu[1], Wenjia Xia[1], Siwei Wang[1], Rong Yin[1], Qin Zhang[1], Lin Xu[1], Hong Fan[3]

[1]Department of Thoracic Surgery, Nanjing Medical University Affiliated Cancer Hospital and Jiangsu Cancer Hospital and Jiangsu Institute of Cancer Research, Jiangsu Key Laboratory of Molecular and Translational Cancer Research, Nanjing 210009, China; [2]Department of Thoracic Surgery, Fujian Cancer Hospital and Fujian Medical University Cancer Hospital and Fujian Provincial Key Laboratory of Tumor Biotherapy, Fuzhou 350014, China; [3]Department of Medical Genetics and Developmental Biology, Medical School of Southeast University, The Key Laboratory of Developmental Genes and Human Diseases, Ministry of Education, Southeast University, Nanjing 210009, China; [4]Institute of Life Sciences, The Key Laboratory of Developmental Genes and Human Disease, Southeast University, Nanjing 210018, China; [5]Department of Pathology, Medical School, Southeast University, Nanjing 210009, China; [6]Department of Pathology, Fujian Cancer Hospital and Fujian Medical University Cancer Hospital, Fuzhou 350014, China

Received April 14, 2019; Accepted April 25, 2019; Epub July 1, 2019; Published July 15, 2019

Abstract: Background: Esophageal cancer (EC) is the fourth most commonly diagnosed cancer in males and the fifth in females in China. Dysregulation methylation of histone is now considered a biomarker for cancer prognosis. Methods: In this study, we focused on exploring the expression patterns of two repressor histone methylation marks, H3K9 dimethylation (H3K9me2) and H3K27 trimethylation (H3K27me3), to provide potential biomarkers for diagnosis and therapies in esophageal squamous cell carcinoma (ESCC). Results: After analyzing the relationship between the expression pattern of H3K27me3 and the clinic-pathological features of ESCC tissues, we found that upregulated H3K27me3 correlated with advanced T stage and N stage. A multivariate Cox regression analysis showed H3K27me3 expression, T stage and N stage were all independent factors for the poor prognosis of ESCC. Therefore, H3K27me3 can be considered an independent factor for predicting the prognosis of ESCC. Conclusions: Chromatin remodeling, especially the methylation of H3, plays a vital role in ESCC development and is a potential therapeutic target.

Keywords: H3K27me3, H3K9me2, ESCC, biomarker

Introduction

Esophageal cancer (EC) is one of the most serious health problems worldwide and ranks eighth in human malignancy. Geographical factors, local culture, and ethnicity play important roles in the incidence rate of EC in different regions [1]. In China, with approximately 477,900 new patients and 375,000 related deaths occurring in 2015, EC is the fourth most commonly diagnosed cancer in males and the fifth in females [2]. Approximately 70% of global EC cases occur in China, with esophageal squamous cell carcinoma (ESCC) being the histopathological form in the vast majority of cases (>90%) [3]. Despite surgery with fast therapeutic effects and adjuvant treatments, the prognosis of ESCC patients still remains poor [4]. Thus, new biomarkers are needed to improve the clinical management of ESCC.

It is understood that epigenetic changes, including DNA methylation and covalent histone modification, are involved in the tumorigenesis and progression of human cancers [5]. Histones undergo posttranslational modifications (e.g. acetylation, methylation, phosphorylation) at their N-terminal tails [6]. Histone lysine methylation is a central factor in such processes as X chromosome inactivation, transcription, DNA

repair and chromosome condensation through breaking chromatin contacts or affecting the recruitment of non-histone proteins to the chromatin. Emerging evidence indicates that the epigenetic alterations play a more fundamental function in the carcinogenesis and development of ESCC [7]. The degree of methylation and the site of the methylation on histone influence transcriptional activity [8]. The methylation at H3K9 and H3K27 is associated with transcriptional repression. According to previous studies, H3K27me3 is typically associated with inactive gene promoters and serves as a suppressor marker [9], and H3K9me2 was reported to be associated with the inflammatory response [10-14]. The statuses of H3K9me2 and H3K27me3 and their clinical implications in ESCC patients are not fully known. The specific histone methylation alterations that occur in ESCC patients remain to be elucidated. To date, the roles of H3K27me3 and H3K9me2 expression in clinical implications for ESCC are rarely studied. In the present study, we aimed to investigate the clinical and prognostic implications of H3K27me3 and H3K9me2 in ESCC patients. Furthermore, we evaluated whether these two histone marks are associated with the clinicopathological features and survival of patients.

Material and methods

Patient information and tissue samples

We used 135 primary ESCC specimens and the corresponding non-tumor esophageal mucosa obtained from patients who underwent surgical treatment for ESCC in the Department of Thoracic surgery of Fujian Provincial Cancer Hospital between 2004 and 2006. None of the patients underwent preoperative adjuvant chemotherapy or radiotherapy. The diagnoses of all the tumors were confirmed by a critical re-examination of the clinical and histopathological findings. Tumor grade and stage were defined according to the 7th edition of the *TNM classification* of the Union for International Cancer Control (UICC) *and* the American Joint Committee on Cancer (AJCC). The patients' clinical characteristics, such as gender, age, tumor location, tumor size, pathological type, pathological grading, and tumor infiltration were obtained from their medical records. All of the patients were followed up for 60 months (average, 32 months). The tumor specimens and corresponding normal esophageal mucosa were collected from paraffin blocks of the 135 primary ESCCs from the Department of Pathology of Fujian Provincial Cancer Hospital.

Construction of tissue microarrays (TMA)

After review and confirmation by the histopathologist, TMA were constructed according to a method described previously [15]. In brief, duplicates of 0.6 mm diameter cylinders were punched from representative tumor areas of individual donor tissue blocks and re-embedded into the paraffin-embedded receiver blocks at a defined position, using a tissue arraying instrument (Beecher Instruments, Silver Spring, MD, USA). We embedded three different cores of each primary ESCC tissue and the corresponding normal esophageal mucosa as determined by routine microscopy on hematoxylin and eosin-stained sections to overcome tumor heterogeneity.

Immunohistochemistry (IHC) staining

IHC staining was used to assess the protein expression on the TMA slides. In brief, TMA sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 3% methanol/H_2O_2 for 15 min. For antigen retrieval, tissue slides were boiled in a 10 mM citrate buffer (pH 6.0) and warmed up to 100°C in a pressure cooker for 5 min. Bull serum albumin (3%) was used to avoid unspecific (hydrophobic) bindings. The primary antibodies, anti-H3K27me3 (1:500; Abcam, Millipore, Temecula, USA) or anti-H3K9me2 (1:200; Abcam, Cambridge, MA, USA), were incubated overnight at 4°C in a moist chamber. Subsequently, the slides were sequentially incubated with secondary antibodies labeled with HRP at a concentration of 1:100 for 30 min at 37°C, then the staining was developed using fresh prepared 3'-3'diaminobenzidine as a chromogenic reagent (DAB, G1211, Wuhan Servicebio, China). The nucleus was counterstained using Meyer's hematoxylin. A negative control was obtained using phosphate buffer saline (PBS) instead of the primary antibody. Two experienced pathologists who were blinded to the patients' clinical information evaluated the samples independently and recorded the IHC results. The staining intensity in the tumor cells was graded as 0~3, and the percentage of H3K27me3/H3K9me2 positive cells was graded according to the proportion of positive cells using a 0~4 grading scale (0: 0%-5%; 1:

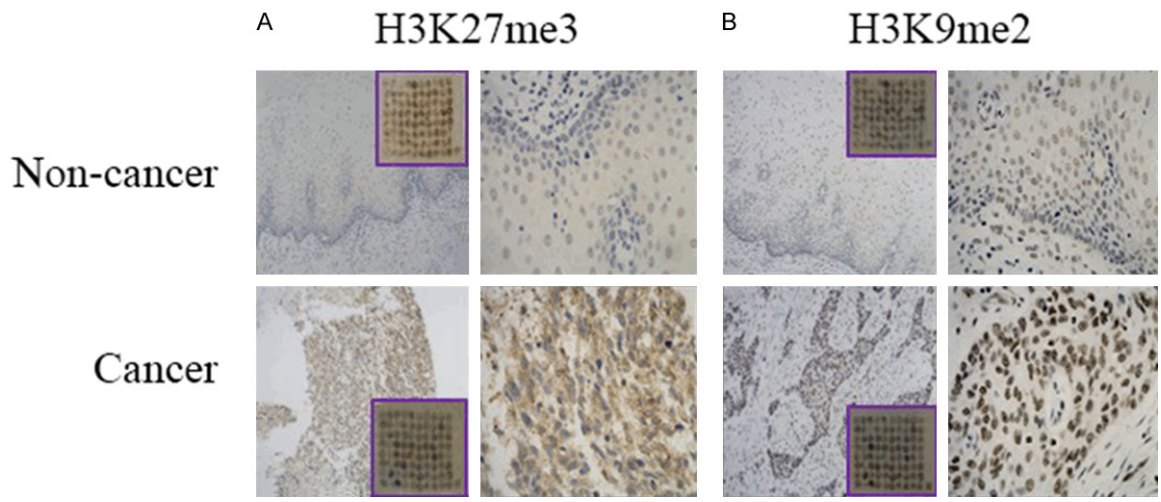

Figure 1. Representative examples of ESCC and matched non-cancerous esophageal mucosa with immunostaining for histone markers including histones H3K9me2 and H3K27me3. Scale bar = 100 μm. (A: H3K27me3, B: H3K9me2).

6%-25%; 2: 26%-50%; 3: 51%-75%; 4: 76%-100%). The final scoring of the H3K27me3/H3K9me2 expression level (positive or negative) was calculated as the sum of both grades (negative: total grade = 0~3; positive: total grade = 4~7).

Statistical analysis

All statistical analyses were carried out using the *SPSS v. 23.0* statistical software packages (Armonk, NY, USA). The correlation between the histone methylation marks expression and the clinic-pathological features of ESCC patients was analyzed using an χ^2 test or Fisher's exact test. For the univariate survival analysis, survival curves were obtained using the Kaplan-Meier method. A Cox proportional hazards model was generated in the multi-factorial survival analysis to identify prognostic factors. An independent Student's *t* test was performed to analyze the statistical significance between the two preselected groups. Survival times are shown in years, but for more exact results they were calculated in months. To show a statistical difference, $P<0.05$.

Results

The expression pattern of H3K27me2 examined by IHC in ESCC TMA

A positive nucleus expression of H3K27me3 and H3K9me2 in the tumor cells and in the non-tumor esophageal mucosa cells was observed (**Figure 1**). We found that the cancer cells showed strong immunostaining for H3K27me3 compared to the non-tumor epithelial cells; furthermore, a significant difference of H3K27me3 expression was observed between the ESCC tissues and the corresponding normal esophageal mucosa group. When examining the correlation between the expression patterns of the histone methylation markers H3K27me3 and clinicopathological factors, we found there was a close correlation between the upregulation of H3K27me3 and T stage, N stage, respectively ($P = 0.034/0.021$, **Table 1**; **Figure 2**).

The expression of H3K9me2 examined by IHC in ESCC tissue TMA

In the present study, the expression of H3K9me2 was examined by IHC in 135 cases of primary ESCC tissues and corresponding normal esophageal mucosa. No statistically significant difference was found in H3K9me2 expression between the tumor group and corresponding normal esophageal mucosa group ($P>0.05$). While we investigated the relationship between H3K9me2 expression patterns and the corresponding clinic-pathological features of ESCC, no significant correlation was found between H3K9me2 expression levels and age, gender, tumor location, tumor differentiation, or AJCC stage (all $P>0.05$, **Table 2**) in the detected TMA. Additionally, there was no

Table 1. The correlation of the H3K27me3 expression pattern with the clinicopathologic features in ESCC (n = 113)

Groups		H3K27me3 expression		Pearson χ^2	*P*-value
		Low	High		
Age	<60	47	25	0.941	0.332
	≥60	25	16		
Gender	Male	52	32	0.465	0.495
	Female	20	9		
Location	Up	18	7	1.005	0.316
	Middle	50	32		
	Low	4	2		
Differentiation	High	16	10	0.163	0.992
	Middle	50	27		
	Low	6	4		
T stage	T1	4	4	3.641	0.034*
	T2	14	4		
	T3	50	28		
	T4	4	5		
N stage	N0	19	13	6.094	0.021*
	N1	23	7		
	N2	25	13		
	N3	5	8		
TNM stage	I	4	3	1.242	0.537
	II	24	10		
	III	44	28		

P<0.05 indicated a significant association. *represents *P*<0.05.

significant difference between H3K9me2 expression in normal esophageal mucosa and the clinic-pathological features of ESCC (data not shown).

Survival analysis

To evaluate the prognostic significance of the expression of H3K9me2 and H3K27me3, a Kaplan-Meier analysis of overall survival (OS) was performed on selected ESCC patients. Our data showed that the median survival time of the 135 patients with ESCC was 32 months. The survival time in patients with H3K27me3-positive ESCC tissues (*P* = 0.0463, **Figure 3A**) but H3K9me2-positive ESCC tissues (*P* = 0.853, **Figure 3B**) was evidently correlated with prognosis. We further evaluated the overall survival of ESCC patients based on the different combination of the protein expression pattern of H3K9me2 and H3K27me3. The average overall survival of patients with high level expressions of both H3K9me2 and H3K27me3 was 36.136 months, which was shorter than the patients with only one protein expression or with negative expressions of both (**Figure 3C**). There was a significant difference between the H3K9me2+ H3K27me3+ expression group and the other groups (*P*<0.05); therefore, the combination of the expression pattern of H3K27me3 and H3K9me2 could provide more accurate information to evaluate the prognosis compared to the individual expressions of H3K27me3 and H3K9me2.

Multivariate Cox regression analysis

A further multivariate analysis of the clinicopathological parameters, including age, gender, location, differentiation, T stage, N stage, TNM stage and the H3k27me3/H3K9me2 expression were performed. As shown in **Table 3**, T stage (HR, 0.318; 95% CI, 0.153-0.662; *P* = 0.045), N stage (HR, 0.203; 95% CI, 0.055-0.754; *P* = 0.017) and H3K27me3 high expression (HR, 2.664; 95% CI, 1.327-5.3474; *P* = 0.006) were important indicators to evaluate the prognosis of patients with ESCC. No significant correlation was found between the H3K9me2 expression and these factors. These data suggested that upregulated H3K27me3 expression, advanced T stage and N stage were all independent factors for the poor prognosis of ESCC. Thus, H3K27me3 could serve as an efficient biomarker in the prediction of ESCC patients' survival.

Discussion

It has been shown that epigenetic modulations of the genome involve histone modifications that alter the chromatin configuration. A decondensed ("open") configuration allows transcription factors access to binding sites, but a condensed ("closed") configuration blocks transcription binding sites. In this way histone modifications can regulate gene transcription [16,

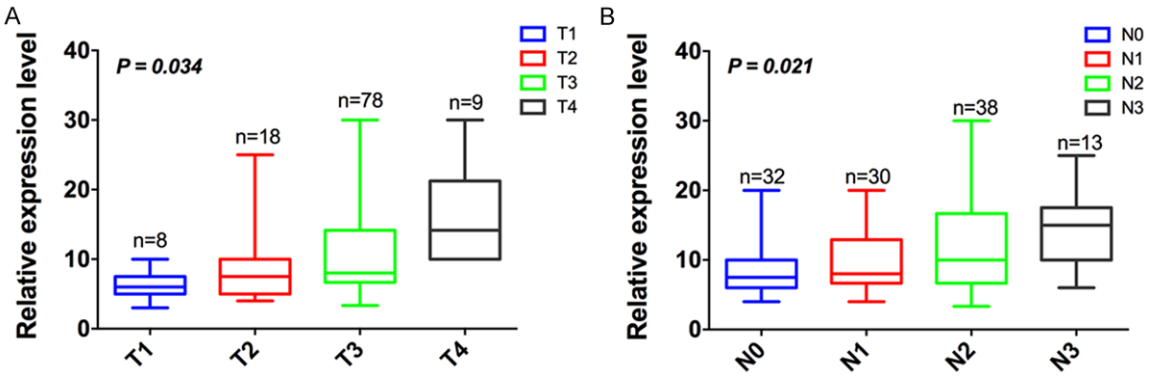

Figure 2. The correlation of H3K27me3 expression levels in ESCC tissues with T-stage and N-stage of ESCC. (A: T-stage, B: N-stage).

Table 2. The correlation between the H3K9me2 expression pattern and the clinicopathologic features in ESCC (n = 92)

Groups		H3K9me2 expression		Pearson χ^2	*P*-value
		Low	High		
Age	<60	31	17	0.926	0.327
	≥60	24	20		
Gender	Male	10	10	1.017	0.313
	Female	45	27		
Location	Up	11	4	1.412	0.494
	Middle	29	21		
	Low	15	12		
Differentiation	High	15	6	4.486	0.106
	Middle	39	26		
	Low	1	4		
T stage	T1	3	2	0.961	0.811
	T2	13	6		
	T3	35	25		
	T4	4	4		
N stage	N0	14	10	1.062	0.786
	N1	15	13		
	N2	19	11		
	N3	7	3		
TNM stage	I	6	1	2.119	0.347
	II	15	11		
	III	34	25		

P<0.05 indicated a significant association.

17]. Evidence shows that histone lysine methylation has been considered an important factor in the process of the tumor development [18, 19]. Studies have reported the relationship between the expression of H3K27me3 and the level of the enhancer of zeste homology 2 (EZH2), which could regulate the histone methyltransferase activity for H3K27me, and which has been positively associated with aggressiveness and poor prognosis in a variety of human malignancies [20-22]. Liu and his colleges reported the expression of EZH2 and H3K27me3 could serve as biomarkers in the prediction of ESCC patients' survival and ESCC metastasis [23]. Similarly, H3K9me2 is directly correlated to the onset and advancement of multiple cancers [24]. However, the correlation between the expression of H3K9me2 and the malignancy of ESCC is rarely reported. Both H3K9me2 and H3K27me3 have been recently reported to be epigenetically altered in human cancers. The concept of molecular staging, which may help to distinguish tumor subtypes by molecularly heterogeneous and different prognoses, has been proposed and investigated in several human cancers.

Investigating the clinical and prognostic implications of H3K27me3 and H3K9me2 in ESCC patients may provide a potential biomarker and target for the treatment of ESCC. To the best of our knowledge, no studies have investigated the combination of H3K9 and H3K27 methylation and their potential impact on ESCC tumorigenesis. Within this study, we recruited 135 cases of ESCC patients to provide both ESCC specimens and corresponding normal esophageal mucosa for immune-histochemical analysis of H3K9me2 and H3K27me3. We demonstrated that the overexpression of H3K27me3 in the

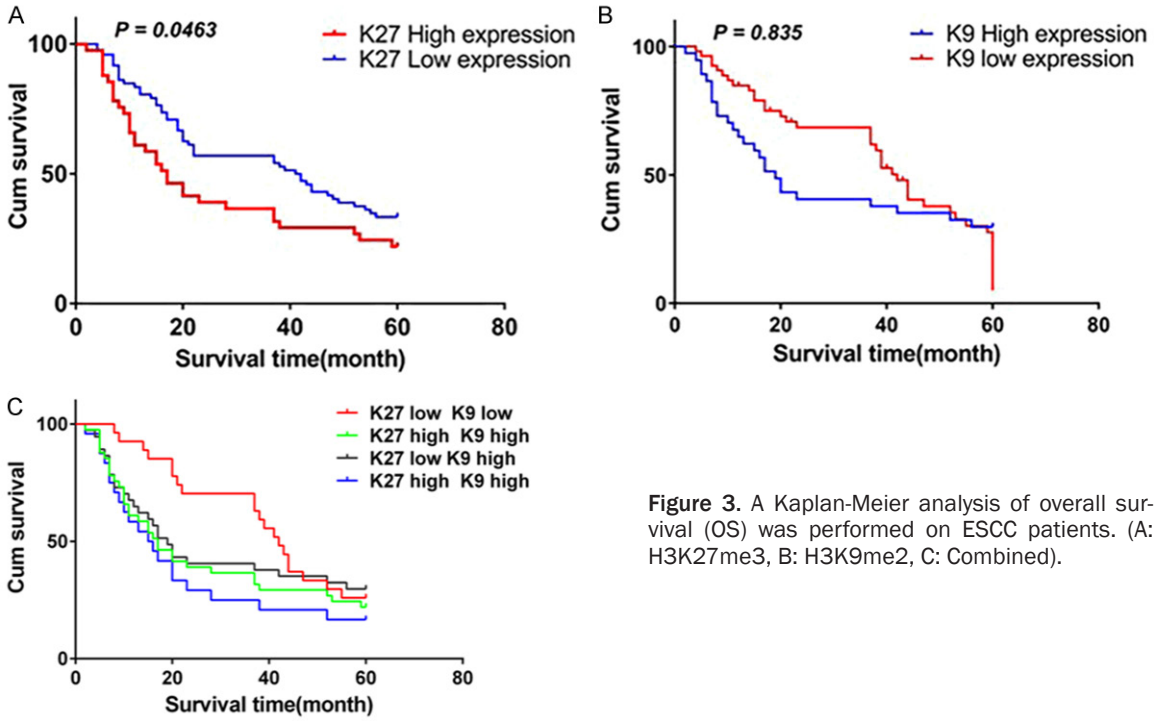

Figure 3. A Kaplan-Meier analysis of overall survival (OS) was performed on ESCC patients. (A: H3K27me3, B: H3K9me2, C: Combined).

Table 3. Analysis of independent correlation factors of ESCC prognosis with Cox multivariate regression analysis (n = 113)

Factor	HR	95% CI	*P*-value
Age (<60 VS ≥60)	0.802	0.418-1.539	0.507
Gender (male VS female)	0.572	0.234-1.345	0.2
Location (up VS middle and low)	1.684	0.814-3.482	0.16
Differentiation (I VS II and III)	0.656	0.342-1.260	0.714
T stage (T1, T2 and T3 VS T4)	0.318	0.153-0.662	0.045*
N stage (N0, N1 and N2 VS N3)	0.203	0.055-0.754	0.017*
H3K27me3 expression (high VS low)	2.664	1.327-5.347	0.006**

P<0.05 indicated a significant association (marked with *), *represents *P*<0.05, **represents *P*≤0.01.

ESCC specimens was associated with some clinic-pathological features of ESCC. However, no significant correlation was found between H3K9me2 expression in ESCC tissues and age, gender, tumor location, tumor differentiation, or AJCC stage. Strikingly, we found that a high H3K27me3 expression was observed in the tumor tissues and correlated with the clinical outcomes for ESCC patients. Nevertheless, H3K9me2-positive ESCC tissues were not significantly correlated with prognosis. When we analyzed the combination of H3K9me2 and H3K27me3, a high level of H3K9me2 and a high level of H3K27me3 in tumors predicted a short survival time. These results indicated that H3K27me3 may serve as a biomarker for discriminating subgroups of patients with more aggressive tumors and thus a poorer prognosis in ESCC.

Recently, histone methyltransferases have been investigated in many studies. G9a (EHMT2) is a histone methyltransferase that dimethylates lysine 9 at histone 3 to reduce transcription activity. H3K27me3, the enhancer of zeste homolog 2, can specially trimethylate lysine 27 on histone H3 of the target gene promoters. EZH2 has been considered a relevant therapeutic target for cancers, and studies show that the inhibition of EZH2 by the small molecular inhibitors or gene knockdown results in a decrease of cancer cell growth and tumor formation [25]. Recently, cancer-related long non-coding RNAs (lncRNAs) have been identified and studied in the field of translational research. Hox transcript antisense intergenic RNA (HOTAIR) is located within the homeobox C (HOXC) gene cluster on chromosome 12 [26, 27]. The repressive histone mark H3K27me3, which occurs through EZH2 enzymatic action and the SUZ12 structural protein, as parts of the PRC2 by the histone demethylation process of the histone mark H3K4me2/3 through LSD1 enzyme [28],

where lncRNA HOTAIR acts as a modular scaffold, seeks a higher ordered lncRNAs epigenetic protein complex, and modifies histone profiles in human normal cells and also in human malignant cells. These processes are involved in tumorigenesis and in the tumor progression of human cancers [28]. The potential mechanism is that cancer-related genes are silenced by these histone methyltransferases. He et al. studied the prognostic impact of H3K27me3 expression on locoregional progression after chemo-radiotherapy in ESCC [29]. Because it is a target of histone methyltransferases, it is important for us to study the expression of histone methylation. This is also the first study that aimed to evaluate the possibility of using H3K9me2 and H3K27me3 as clinical indicators of disease progression as well as a prognostic marker for ESCC patients.

Epigenetic aberrations, such as histone modification, have the ability to regulate the expression of oncogenes or the repression of tumor suppressor genes. Therefore, these modified histone proteins are potential candidates in the investigation of cancer pathogenesis and progression. It is known that there are subgroups of patients characterized with molecularly heterogeneous and different clinical prognosis in ESCC. Therefore, it is crucial to determine distinctive molecular biomarkers in order to discriminate among subgroups of patients and to direct individual therapeutic interventions. Our study provides the evidence that H3K9me2 and H3K27me3 cooperate in ESCC and that the upregulation of these two markers predicts poor prognosis. These results may enlighten the understanding of the epigenetic mechanisms involved in tumors and also merits the investigation of global histone modification in ESCC.

Acknowledgements

This work was supported by the Innovation Capability Development Project of Jiangsu Province (BM2015004), the Project of Jiangsu Provincial Medical Talent (ZDRCA2016033), and the Key Project of Cutting-edge Clinical Technology of Jiangsu Province (BE2017759, BE2016797).

Disclosure of conflict of interest

None.

Abbreviations

EC, Esophageal cancer; ESCC, esophageal squamous cell carcinoma; OS, overall survival; TMA, tissue microarrays; PBS, phosphate buffer saline; EZH2, enhancer of zeste homology 2; lncRNAs, long non-coding RNAs; HOTAIR, hox transcript antisense intergenic RNA; HOXC, homeobox C.

Address correspondence to: Lin Xu, Department of Thoracic Surgery, Nanjing Medical University Affiliated Cancer Hospital and Jiangsu Cancer Hospital and Jiangsu Institute of Cancer Research, Cancer Institute of Jiangsu Province, Nanjing 210009, China. E-mail: xulin83cn@gmail.com; Hong Fan, Department of Medical Genetics and Developmental Biology, Medical School of Southeast University, Nanjing 210009, China. E-mail: fanh@seu.edu.cn

References

[1] Schrump DS, Nguyen DM. Novel molecular targeted therapy for esophageal cancer. J Surg Oncol 2005; 92: 257-261.
[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
[3] Xu Y, Yu X, Chen Q, Mao W. Neoadjuvant versus adjuvant treatment: which one is better for resectable esophageal squamous cell carcinoma. World J Surg Oncol 2012; 10: 173.
[4] Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med 2003; 349: 2241-2252.
[5] Esteller M. Cancer epigenomics: DNA methylomes and his-tone-modification maps. Nat Rev Genet 2007; 8: 286-298.
[6] Lund AH, van Lohuizen M. Epigenetics and cancer. Genes Dev 2004; 18: 2315-2335.
[7] Lin DC, Wang MR, Koeffler HP. Genomic and epigenomic aberrations in esophageal squamous cell carcinoma and implications for patients. Gastroenterology 2018; 154: 374-389.
[8] Bartke T, Vermeulen M, Xhemalce B, Robson SC, Mann M, Kouzarides T. Nucleosome-interacting proteins regulated by DNA and histone methylation. Cell 2010; 143: 470-484.
[9] Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. Cell 2007; 129: 823-837.
[10] De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L, Bucci G, Caganova M, Notarbartolo S, Casola S, Testa G, Sung WK, Wei CL, Natoli G. Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. EMBO J 2009; 28: 3341-52.

[11] Chen X, El Gazzar M, Yoza BK, McCall CE. The NF-kappaB factor RelB and histone H3 lysine methyltransferase G9a directly interact to generate epigenetic silencing in endotoxin tolerance. J Biol Chem 2009; 284: 27857-65.
[12] De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. Cell 2007; 130: 1083-94.
[13] Chan C, Li L, McCall CE, Yoza BK. Endotoxin tolerance disrupts chromatin remodeling and NF-kappaB transactivation at the IL-1beta promoter. J Immunol 2005; 175: 461-8.
[14] Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature 2007; 447: 972-8.
[15] Southgate J, Hutton KA, Thomas DF, Trejdosiewicz LK. Normal human urothelial cells in vitro: proliferation and induction of stratification. Lab Invest 1994; 71: 583-594.
[16] Yu Y, Zeng P, Xiong J, Liu Z, Berger SL, Merlino G. Epigenetic drugs can stimulate metastasis through enhanced expression of the pro-metastatic Ezrin gene. PLoS One 2010; 5: e12710.
[17] Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003; 33 Suppl: 245-254.
[18] Yi X, Jiang XJ, Li XY, Jiang DS. Histone methyltransferases: novel targets for tumor and developmental defects. Am J Transl Res 2015; 7: 2159-75.
[19] Soejima H, Nakagawachi T, Zhao W, Higashimoto K, Urano T, Matsukura S, Kitajima Y, Takeuchi M, Nakayama M, Oshimura M, Miyazaki K, Joh K, Mukai T. Silencing of imprinted CDKN1C gene expression is associated with loss of CpG and histone H3 lysine 9 methylation at DMR-LIT1 in esophageal cancer. Oncogene 2004; 23: 4380-8.
[20] Mimori K, Ogawa K, Okamoto M, Sudo T, Inoue H, Mori M. Clinical signicance of enhancer of zeste homolog 2 expression in colorectal cancer cases. Eur J Surg Oncol 2005; 31: 376-80.
[21] Sudo T, Utsunomiya T, Mimori K, Nagahara H, Ogawa K, Inoue H, Wakiyama S, Fujita H, Shirouzu K, Mori M. Clinicopathological signicance of EZH2 mRNA expression in patients with hep-atocellular carcinoma. Br J Cancer 2005; 92: 1754-1758.
[22] Collett K, Eide GE, Arnes J, Stefansson IM, Eide J, Braaten A, Aas T, Otte AP, Akslen LA. Expression of enhancer of zeste homologue 2 is significantly associated with increased tumor cell proliferation and is a marker of aggressive breast cancer. Clin Cancer Res 2006; 12: 1168-1174.
[23] Liu F, Gu L, Cao Y, Fan X, Zhang F, Sang M. Aberrant overexpression of EZH2 and H3K27me3 serves as poor prognostic biomarker for esophageal squamous cell carcinoma patients. Biomarkers 2016; 21: 80-90.
[24] Mayr C, Helm K, Jakab M, Ritter M, Shrestha R, Makaju R, Wagner A, Pichler M, Beyreis M, Staettner S, Jaeger T, Klieser E, Kiesslich T, Neureiter D. The histone methyltransferase G9a: a new therapeutic target in biliary tract cancer. Hum Pathol 2018; 72: 117-126.
[25] Yan KS, Lin CY, Liao TW, Peng CM, Lee SC, Liu YJ, Chan WP, Chou RH. EZH2 in cancer progression and potential application in cancer therapy: a friend or foe. Int J Mol Sci 2017; 18: 1172.
[26] Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding RNAs and cancer: a new frontier of translational research. Oncogene 2012; 31: 4577-4587.
[27] Wahlestedt C. Targeting long non-coding RNA to therapeutically upregulate gene expression. Nat Rev Drug Discov 2013; 12: 433-446.
[28] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010; 329: 689-693.
[29] He LR, Liu MZ, Li BK, Rao HL, Liao YJ, Guan XY, Zeng YX, Xie D. Prognostic impact of H3K27me3 expression on locoregional progression after chemoradiotherapy in esophageal squamous cell carcinoma. BMC Cancer 2009; 9: 461.